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Environmental Effects of Dredging Technical Notes

Evaluation of Sediment Genotoxicity: Workshop Summary and Conclusions

Purpose

This Technical Note summarizes the proceedings of a workshop that was held March 6-8, 1990, at the Environmental Laboratory, US Army Engineer Waterways Experiment Station. The purpose of the workshop was to gain guidance from recognized authorities for the development of sediment bioassays of genotoxicity, that is, mutagenicity, carcinogenicity, immunotoxicity, teratogenicity, and histopathologic potential. The conclusions of the workshop are being used to identify existing genotoxicity bioassays that show promise for application in evaluating sediments, to recommend modifications for testing sediments, and to help direct subsequent research and development of bioassays of genotoxicity by the US Army Corps of Engineers.

Background

The US Army Corps of Engineers (USACE) is responsible for maintaining, extending, and improving the Nation's waterways. In carrying out its mission, the USACE now dredges or regulates the dredging of more than 230 million cubic yards (cu yd) in maintenance and about 70 million cu yd in new dredging operations annually, at a cost of about $500-600 million. Additionally, about 150 million cu yd dredged by others are regulated by permits issued by the USACE. Regulatory responsibilities of the USACE in this context involve the annual review of 10,000-30,000 dredge and fill permit applications nationwide. The authority of the USACE stems from Section 10 of the Rivers and Harbors Act of 1899, Section 404 of the Clean Water Act (Public Law 92-500, as amended), and Section 103 of the Marine Protection, Research, and Sanctuaries Act ("Ocean Dumping Act," Public Law 92-532, as amended). Compliance with both laws involves, among

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other things, the avoidance of "unacceptable adverse impacts," and Section 103 specifically prohibits "known *carcinogens, mutagens, or teratogens or materials suspected to be carcinogens, mutagens, or teratogens by responsible scientific opinion.*" These substances are prohibited under Section 227.6, *Constituents prohibited as other than trace contaminants.* In Section 103, constituents are identified as trace contaminants if, as a result of bioassays, there is *"reasonable assurance . . . that when the materials are dumped, no significant undesirable effects will occur due either to chronic toxicity or to bioaccumulation. . . ."* The nature of tests that are mandated under Section 103, are clearly effects based. Chemical inventories of sediments can be included, but regulatory decisions regarding trace contaminants must be based on the results of bioassays.

About 5-10 percent of the maintenance and a much smaller part of the new-work dredged material is considered contaminated and is unacceptable for unrestricted open-water disposal. Both law and the public interest require that these contaminated sediments be identified and disposed of in the most environmentally responsible manner possible. In the regulatory evaluation of contaminated sediments for dredging and disposal, bioassays are used to assess acute toxicity and bioaccumulation. As yet, no bioassay methods exist that are recognized as appropriate for detection of carcinogenic, mutagenic, or teratogenic effects on aquatic organisms of dredged material proposed for open-water disposal.

Headquarters, US Army Corps of Engineers has authorized the start of a new work unit under the Long-Term Effects of Dredging Operations (LEDO) Program to develop dredged sediment genotoxicity bioassays for application in regulating disposal operations. In this context the term "genotoxicity" is used broadly to encompass all carcinogenic, mutagenic, or teratogenic effects of chemically contaminated sediments in aquatic biota, whether mediated through genetic or epigenetic mechanisms.

The purpose of the Genotoxicity Workshop was to provide guidance to the USACE for developing bioassays addressing this problem. For implementation, bioassays must be predictive, interpretable, reliable, and economical. In regulatory testing of contaminated sediments, bioassays have until now involved only whole organisms. Bioassays for sediment genotoxicity (as defined), it is anticipated, may properly include the use of biomarkers. Bioassays can be operationally defined as the exposure of a biological system (whole organisms or tissues) to test conditions to find changes caused by the test conditions. "Biomarkers" are indicators of chemical effects on biological functions that are observed at suborganismal levels. Bioassays and biomarkers may be recommended in a suite of related tests that together imply the probability of genotoxic effects. In order to be most persuasive, relationships must be describable in terms of mechanisms of action whenever possible, as opposed to being merely correlating. Ideally, the probability of effects such as cancer or lethal defects at birth will be established by the early observation of biomarkers.

Workshop participants were selected for their technical expertise in various phases of genotoxicity, as well as their familiarity with aquatic sediment processes

and the regulatory community. All are recognized as experts in their fields. A list of the participants with their affiliation is included in Table 1. Several Corps personnel with expressed interest in genotoxicity were invited to participate in the workshop. Those who attended are also listed in Table 1.

This Technical Note summarizes problems identified during the workshop and the consensus regarding the best way to address them. The participants also contributed written submissions that address specific applications in sediment genotoxicity testing. These will be included in a Miscellaneous Paper to be published in 1991.

Additional Information

This Technical Note was written by Mr. Francis J. Reilly, Jr., Mr. Victor A. McFarland, Ms. Joan U. Clarke, Ms. A. Susan Jarvis, Dr. Robert B. Spies, and Dr. Richard F. Lee. For additional information, contact Mr. Reilly, (601) 634-4148, or Mr. McFarland, (601) 634-3721, or the manager of the Environmental Effects of Dredging Programs, Dr. Robert M. Engler, (601) 634-3624.

Sediment Genotoxicity Workshop

The workshop was moderated by Mr. Francis J. Reilly, Jr., who introduced the speakers. The workshop participants were welcomed to the Waterways Experiment Station (WES) by COL Mack Goldman, Deputy Commander and Director of WES. Mr. Thomas Patin, Program Manager, Dredging Operations Technical Support Program, gave a brief presentation on the research and field demonstrations aspects of the Environmental Effects of Dredging Programs. The need for genotoxicity testing from a regional Corps of Engineers perspective was presented by Dr. Lloyd Saunders, Chief, Contaminant Mobility and Regulatory Criteria Group (CMRCG). Dr. Saunders ended his presentation with some questions developed jointly with Dr. Thomas Wright, also of the CMRCG:

- Do tumors in fish pose human health problems?
- What is/are the relationship(s) between sediments and tumors?
- Are there effects at the population level of organization?
- How many tumors are too many?

The research perspective of the US Environmental Protection Agency (EPA) was presented by Dr. Susan Cormier of the EPA Office of Research and Development, Environmental Monitoring Systems Laboratory (EMSL). The focus of EMSL is ecological, not human health effects. Ecological events can occur within a toxicological hierarchy of organization that includes chemical loading of contaminants into sediments, bioavailability of contaminants, possible biotransformation of the contaminants, biochemical and cellular changes due to

the contaminants or the transformed contaminants, and certain biologically meaningful endpoints such as reproductive impairment or death. These biologically meaningful endpoints can result in ecological damage by affecting population densities of species and effecting changes at the community level of biological organization. Bioindicators can be defined as indicative of change or degradation at any level of the hierarchy. Organism level effects (for example, reproductive effects and tumors) can be monitored by the use of sentinel species or biomonitors and can signal habitat degradation. However, these changes are gross in nature. What is needed is a suite of indicators of change at the cellular or biochemical level of organization, namely, biomarkers.

Different types of biomarkers include those that indicate general health, those that indicate changes in specific organismic functions, and those that indicate nonspecific exposure to chemicals or damage due to specific toxicants. The EPA expects to use a suite of biomarkers and is currently investigating the usefulness of several of these. The tests that are selected will have broad responsiveness and sensitivity, with applicability to a wide range of species. They must also be fast, inexpensive, and reproducible.

Mr. Victor McFarland, Team Leader of the Aquatic Contaminants Team, CMRCG, presented the Corps research perspective regarding genotoxicity testing. The required attributes of a genotoxicity assay for USACE regulatory use include the ability to clearly relate the genotoxicity to sediment-associated contamination. The tests must indicate ecological relevant effects. They must also share the attributes of reliability, reproducibility, cost effectiveness, and ease of use.

A suite of biomarkers were discussed by the workshop participants. Table 2 gives the names of the genotoxicity biomarkers or assays that were discussed. The biomarkers or assays were ranked by consensus in three categories. Ease of use was discussed for each biomarker or assay and a ranking of high, medium, or low ease of use was ascribed to each. The biomarkers' ecological relevance and relevance to the USACE mission requirements regarding clear relationships to sediment toxicity were ranked as either of high, medium, or low relevance. The biomarkers and assays were also ranked on a cost-per-sample basis as either inexpensive or expensive. Comments were allowed for each bioassay or biomarker, and it is here that the developmental stage of each was discussed. Very few of the assays are ready to be tested for use in the regulatory arena, and none are ready for use without some development and interpretive guidance. The usefulness of each biomarker or assay for specific organisms was also discussed.

Conclusions

After the second day of the workshop, four participants met to draft a consensus paper outlining the results, conclusions, and recommendations of the workshop. The authors were: Dr. Richard Lee, Mr. Victor McFarland, Mr. Francis J. Reilly, Jr., and Dr. Robert Spies. The consensus draft was presented to the entire workshop on the third day of the workshop and corrections and additions were made and have been incorporated in the following paragraphs.

Biomarkers and Bioassays

To achieve the workshop objectives, the group decided that it will be necessary to develop biomarkers of exposure, integrators of effects at higher levels of biological organization, and general indicators of genotoxic potential. After evaluation of a number of biomarkers and bioassays, the participants agreed that the following showed the most potential for development in the framework of a four- to five-year program:

Biomarkers of Exposure. None of these taken singly is sufficient to prove causality of tumor development or other genotoxic effects, but all are indicative of exposure to potentially genotoxic chemicals. There are three main groups of biomarkers of exposure: proteins/enzymes, bile metabolites, and DNA adducts. The induction (increased production) of detoxifying proteins such as cytochrome P-450 or P-450-dependent enzymes (AHH, EROD, and ECOD) can be measured either via specific protein assays, antibody reactions, or by measurement of messenger RNA (mRNA). Certain genotoxic agents can be easily detected as metabolized compounds excreted in the bile of exposed organisms. Reaction products formed from genotoxic chemicals and nucleic acids can also be detected.

General Indicators of Genotoxicity. Changes in an organism's genetic integrity due to exposure to genotoxicants can be measured by mutations, chromosomal abnormalities, or DNA strand breaks. A host of bioassays exist that can be performed in either the test tube (*in vitro*), or in an intact organism (*in vivo*). Those that may be applicable to sediment genotoxicity testing are discussed in the following paragraphs.

Methods that detect chemically caused mutations may be either *in vitro* or *in vivo*: *In vitro* methods that have been applied to sediment contaminants include the Chinese Hamster Ovary (CHO) test, the Syrian Hamster Embryo (SHE) test, and the Ames Salmonella Test. *In vivo* bioassays for mutagenicity that may be applicable to contaminants in sediments are measurements of oncogene formation in an organism following exposure (for example, K-ras).

Cytogenetic techniques are methods that detect damage done to the chromosomes of cells. Chromosome anaphase or telophase abnormalities, micronuclei formation, or sister chromatid exchange can be applied either *in vitro* using CHO or SHE cell lines, or *in vivo* using medaka or other fishes, or invertebrates such as sea urchins or polychaete worms.

Methods that detect DNA damage are mainly *in vivo*, but are indicative of direct genotoxicity. These include adduct formation, minor nucleotide formation, DNA strand breaks, and unscheduled DNA synthesis (UDS) or repair.

Integrators of Genotoxic Effects. None of the biomarkers or subchronic bioassays are sufficient in themselves to predict tumors or terats. Also, many of the chemicals that are implicated in the etiology of cancer or birth defects and that may be sediment contaminants are not initiators (direct acting on genetic material). Some of the workshop scientists estimated that up to 40 percent of

carcinogens in sediments are promoters that create the conditions necessary for a genetically damaged cell to become neoplastic (for example, dioxin and PCBs). For this reason the participants agreed that integrated assays of genotoxic effects should be part of the testing strategy.

Bioassays of whole animal carcinogenesis and gross abnormalities in the embryonic development of exposed organisms should be included in the testing protocol. To date, most existing work on tumorogenesis and teratogenesis has been accomplished using vertebrates. Therefore, if scientifically valid assays of integrated genotoxic effect are to be obtained in a timely manner, the ultimate objective of sediment genotoxicity testing must involve the development of a suitable fish model for direct assessment of tumor production and developmental abnormalities. The much more rapid and less costly biomarkers and subchronic bioassays listed above should at the same time be tested for responsiveness to sediment chemical contaminants. As a data base consisting of results of the short-term and the chronic test methods develops, establishing predictive relationships will be possible. Eventually, a testing suite consisting of certain biomarkers and subchronic bioassays will evolve as acceptable predictors of the real potential for genotoxic effects.

Test Species

Cytogenetic and metabolic studies, studies of neoplasm development, and studies of development abnormalities will all require early decisions on suitable species. The group thought that it would be desirable to first choose a single species that could be used throughout the country, followed by later development of regional species. Consideration was made of animals living in the water column versus benthic infaunal organisms. It was agreed that fish models are the best subjects for genotoxicity testing due to the body of literature that already exists. The Japanese medaka shows great promise because of the large amount of work that has been done to date. Medaka is very susceptible to neoplasm production as a result of exposure to carcinogens and mutagens. Additionally, the medaka can easily be cultured under a variety of environmental regimes and varying salinities.

A major disadvantage is the small size of the fish for carrying out some of the proposed biomarker assays. However, it was agreed that a first task is to determine whether a fish in the laboratory can be made to develop cancer from exposure to contaminants in sediment. This must be done reliably and consistently, or the subchronic bioassays and biomarkers will be of little use. It is realized that a larger species will be needed for other types of endpoints.

Invertebrates do not appear to be suitable for cancer studies, but may be useful for other purposes. The polychaete, *Neanthes arenaceadentata*, has been used in cytogenetic studies, and oyster larvae have been used to detect chemically induced developmental abnormalities. Invertebrate genotoxicity testing is much less developed than are tests using fish.

Research and Implementation Strategy

Many biomarker assays are not conducive to being carried out with whole sediment. Thus, extracts of sediments are often used for these tests. Extracting the genotoxic agents from sediments eliminates any possibility of making an assessment of the bioavailability of such substances. Substances that are genotoxic when extracted may not be genotoxic in the real world. Therefore, positive indications in these assays should not be the sole basis for regulatory decisions, but they should be used in combination with other more ecologically relevant whole sediment exposures to indicate potential for effects.

Various types of extraction procedures will have to be examined as surrogates for bioavailability of genotoxic compounds. Other exposure routes should be investigated. An example of the above might be exposure of benthic infauna (for which no biomarkers are available) to contaminated sediments, followed by their use as a food source for fish (for which there are ample biomarkers of genotoxicity).

Once the appropriateness of the fish models has been confirmed for some of the genotoxic endpoints with polluted sediments, for example, tumor formation, then the strategy should be to determine which of the remaining biomarkers in the above list are responsive to such exposures.

Further research should then be limited to those biomarkers that have responded well to the trial testing as discussed above. However, it should be recognized that new biomarkers will arise and should be tested. New techniques will probably become available because of the speed with which molecular biology is developing. A data base will have to be developed for the selected biomarkers and assays of genotoxicity prior to interpretive guidance. The data base should show a linkage of the effects measured with sediment contamination, as well as dose-responsiveness.

Table 1

Technical Participants

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Dr. Richard Di Giulio, Duke University, School of Forestry and Environmental Studies, Durham, NC 27706

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Table 2

Working List of Biomarkers Discussed at the Genotoxicity Workshop

Biomarker	Relevance*	Ease of Use**	Cost †	Comments
Bile metabolites	M	H-M	I	Large vertebrate
DNA adducts	H	L	E	Not in all organisms
Peroxisome proliferation	L	H	I	Needs development
Minor nucleotides		H	I	Needs development
DNA flow cytometry	H	H	E	High initial cost
Chromosome aberrations	H	M	E	Depending on species, time consuming, problems with interpretation
Oncogenes	H	M	E	Available now in some fish, for example, flounder
Single strand breaks	H	H	I	Available now in medaka
EROD (ethoxy resorufin oxygen deethylase)	M	H	I	Fish sensitive, but negative results are hard to interpret
Glutathione-S-transferase	L	H	I	Low response except in medaka, overlap with EROD
P450	L	H	I	Overlap with EROD

(Continued)

* Biomarkers were ranked on their applicability to sediment testing as well as their ecological relevance; H = highly relevant, M = moderately relevant, L = little relevance.

** Biomarkers were ranked on their projected ease of use by contract laboratory personnel; H = easy to use, M = moderately difficult, L = very difficult.

† Biomarkers and assays were ranked on the expense of performing the assay. Taken into account were cost of startup, and quality control considerations (for example, required replicates for reproducibility).

Table 2 (Concluded)

Biomarker	Relevance	Ease of Use	Cost	Comments
Oxyradical Scavenging Enzyme	L	M	E	Needs development
Stress Protein	L	M	E	Needs development
Histopathology	M	M	E	Important in liver
Embryological Development				
Gross	H	H	I	Inexpensive
Fine	H	H	E	Very expensive
CHO	H	H	I	Requires sediment extract
SHE	H	H	I	Requires sediment extract
AMES/HPTLC (High Performance Thin-Layer Chromatography)	H	H	I	Requires sediment extract, but identifies chemical class of mutagen
Fluctuation	M	H	I	Field or lab applicable
Fish Carcinogenesis	H	H	E	At least 3 month exposure required, does not work for aromatic amines
UDS (Unscheduled DNA Synthesis)	Unknown	H	E	Needs development
Macrophage*				
Other Immunocompetence*				

* Should be considered outside of genotoxicity.